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IS*1R*-Mediated Plasticity of IncL/M Plasmids Leads to the Insertion of *bla*$_{OXA-48}$ into the *Escherichia coli* Chromosome

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The OXA-48 carbapenemase is mainly encoded by ~62-kb IncL/M plasmids. However, chromosome-mediated genes have been observed in *Escherichia coli* isolates. In this work, we investigated the genetic environment of OXA-48 in members of the family *Enterobacteriaceae* ($n = 22$) to understand how the OXA-48-encoding gene is transferred into the *E. coli* chromosome. The OXA-48-encoding gene was located within intact Tn*1999.2* transposons in the ~62-kb plasmids or within a truncated variant of Tn*1999.2* for the OXA-48-encoding genes located in the chromosomes of *E. coli* bacteria. The analysis of the Tn*1999.2* genetic environment revealed an inverted orientation of the transposon in five ~62-kb plasmids (5/14 [35%]) and in all chromosome inserts ($n = 8$). The sequencing of pRA35 plasmid showed that this orientation of Tn*1999.2* and the acquisition of an IS*1R* insertion sequence generated a 21.9-kb IS*1R*-based composite transposon encoding OXA-48 and designated Tn*6237*. The sequencing of a chromosomal insert encoding OXA-48 also revealed this new transposon in the *E. coli* chromosome. PCR mapping showed the presence of this element in all strains harboring an OXA-48-encoding chromosomal insert. However, different insertion sites of this transposon were observed in the *E. coli* chromosome. Overall, these findings indicate a plasticity of the OXA-48 genetic environment mediated by IS*1R* insertion sequences. The insertion sequences can induce the transfer of the OXA-encoding gene into *E. coli* chromosomes and thereby promote its persistence and expression at low levels.

Carbapenems are broad-spectrum antibiotics that constitute the last-line therapeutic option available to treat infection caused by multidrug-resistant members of the family *Enterobacteriaceae* (1). However, owing to the emerging resistance to carbapenems worldwide, the antimicrobial activity of these drugs is no longer guaranteed (2). An underlying mechanism is the acquisition of carbapenem-hydrolyzing β-lactamases encoded by genes located on mobile genetic supports, which facilitate their diffusion among bacteria (3).

The OXA-48 carbapenemase was initially identified from a *Klebsiella pneumoniae* isolate in Turkey (4). It then spread to various *Enterobacteriaceae* species especially throughout the southern Mediterranean area and in Europe (4, 5). The OXA-48-encoding genes (*bla*$_{OXA-48}$) are mostly found in *K. pneumoniae* and, to a lesser extent, in *Escherichia coli* and *Enterobacter* spp. (5). Their emergence is mediated by the rapid spread of strains containing broad-host-range conjugative IncL/M plasmids (6–8) harboring *bla*$_{OXA-48}$ located within the Tn*1999*-type composite transposon (6, 9–11). The plasmid implicated in the diffusion of *bla*$_{OXA-48}$ was a widespread 62-kb IncL/M plasmid (6). Derivatives have also been reported (7, 8). However, *bla*$_{OXA-48}$ was observed in *E. coli* chromosomes, including in a strong killer strain (12).

The aim of this work was to investigate the genetic environment of *bla*$_{OXA-48}$ in order to understand how this gene is transferred into the *E. coli* chromosome.

MATERIALS AND METHODS

Bacterial isolates. Twenty-two *Enterobacteriaceae* strains with reduced susceptibility or resistance to ertapenem and/or imipenem were used in the study (Table 1). Nineteen strains were recovered from clinical samples of patients hospitalized in Nini Hospital, Tripoli, Lebanon, between January 2008 and December 2012. The other three strains were collected in December 2012 during a study investigating intestinal carriage in healthy children. The strains were identified using the matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) Vitek microbial identification system (bioMérieux, La Balme, France). Rifampin-resistant *E. coli* C600 was used for mating-out assays.

Determination of genetic relatedness and *E. coli* phylogroup. The genetic relatedness of the strains was determined by multilocus sequence typing (MLST). Allelic profiles and sequence types (STs) were assigned using the MLST schema of Diancourt et al. (13) and Wirth et al. (14) for *K. pneumoniae* and *E. coli* isolates, respectively.

Molecular characterization of *bla*$_{OXA-48}$ and the associated transposon. The OXA-48-encoding genes were detected by PCR amplification as previously reported (12). The genetic environment of the *bla*$_{OXA-48}$ gene was further investigated by PCR and sequencing using specific primers to map the transposon composites Tn*1999* to Tn*1999.4* (6, 9–11) (Table 2). The orientation of these transposons in their genetic environments was investigated by PCR and sequencing using combinations of primers targeting *bla*$_{OXA-48}$ (5′-AATACACGCATAACGTCCCC-3′ and 5′-GCCATCACAAAAGAAGTGCTC-3′), *lysR* (5′-GCTGCAAATAGCATCATACC-3′), *orf67* (5′-CAGCCAGAATAAGAGCAATC-3′), and *pemI* (5′-TGACCATGCCAACTTCATT-3′). The sequences of PCR products were established on both strands by dideoxy chain terminator using an Applied Biosystems sequencer.

Plasmid analysis. The transferability of the *bla*$_{OXA-48}$ gene was studied by a mating-out assay. Selection was performed on agar plates supplemented with ticarcillin (32 μg/ml) and rifampin (300 μg/ml). The plasmid content of bacteria and the size of plasmids were determined using plasmid DNA extracted by the method of Kado and Liu (15) with the plasmids Rsa (39 kb),

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TABLE 1 Phenotypic and genotypic characteristics of OXA-48-producing isolates

Isolate	ST[a] (*E. coli* phylogroup)	Clinical sample[b] (yr)	OXA-48 genetic environment				
			Transposon	PCR result[c]			Genetic support[d]
				parA	*repA*	*traU*	
E. coli isolates							
EC37	88 (A)	Stool[e] (2012)	Inverted ΔTn*1999.2*	+	−	−	Chrom
EC267	88 (A)	Sputum (2012)	Inverted ΔTn*1999.2*	+	−	−	Chrom
EC264	227 (A)	Urine (2012)	Inverted ΔTn*1999.2*	+	−	−	Chrom
EC15	127 (B2)	Sputum (2011)	Inverted ΔTn*1999.2*	+	−	−	Chrom
EC8	131 (B2)	Urine (2012)	Inverted ΔTn*1999.2*	+	+	+	Chrom
EC254	38 (D)	Urine (2012)	Inverted ΔTn*1999.2*	+	−	−	Chrom
EC265	38 (D)	Pus (2012)	Inverted ΔTn*1999.2*	+	−	−	Chrom
EC49	38 (D)	Stool[e] (2012)	NT[f]	+	−	−	Chrom
EC253	617 (A)	Urine (2012)	Tn*1999.2*	+	+	+	62kb
EC269	617 (A)	Urine (2012)	Tn*1999.2*	+	+	+	62kb
EC119	3877 (A)	Stool[e] (2012)	Tn*1999.2*	+	+	+	62kb
EC260	1711 (B1)	Urine (2012)	Tn*1999.2*	+	+	+	62kb
K. pneumoniae isolates							
KP78	1157	Blood (2012)	Tn*1999.2*	+	+	+	62kb
KP87	1156	Pus (2012)	Tn*1999*	+	+	+	62kb
KP34	45	Urine (2011)	Tn*1999.2*	+	+	+	62kb
KP9	866	Urine (2009)	Inverted Tn*1999.2*	+	+	+	62kb
KP26	866	Urine (2008)	Tn*1999.2*	+	+	+	62kb
E. aerogenes EA2		Pus (2012)	Inverted Tn*1999.2*	+	+	+	62kb
E. cloacae isolates							
ECL6		Urine (2010)	Inverted Tn*1999.2*	+	+	+	62kb
ECL8		Urine (2009)	Tn*1999.2*	+	+	+	62kb
C. koseri CK8		Urine (2011)	Inverted Tn*1999.2*	+	+	+	62kb
R. planticola RA35		Pus (2011)	Inverted Tn*1999.2*	+	+	+	62kb

[a] Sequence types for *E. coli* and *K. pneumoniae* isolates.
[b] Clinical sample that the strain was isolated from.
[c] PCR result for "pOXA-48a genes."
[d] Chrom, chromosome-mediated OXA-48; 62kb, ~62-kb IncL/M plasmid encoding OXA-48.
[e] Fecal carriage in children having no known contact with a hospital.
[f] NT, nontypeable Tn*1999*-type environment.

TP114 (61 kb), pCFF04 (85 kb), and pCFF14 (180 kb) as standards. Plasmid restriction analysis was performed from DNA extracted by alkaline lysis and digested with EcoRI and HindIII restriction endonucleases (Boehringer Mannheim, Meylan, France). PCR-based replicon typing (PBRT) was used to identify plasmid incompatibility groups in transconjugants (16). The *repA*, *traU*, and *parA* genes were detected by PCR to relate the OXA-48-encoding plasmids to the pOXA-48a IncL/M plasmid as previously described (6).

Chromosome analysis. The chromosomal location of the bla_{OXA-48} gene was investigated by pulsed-field gel electrophoresis (PFGE) using the I-CeuI endonuclease and hybridization with probes specific for the 16S rRNA genes, bla_{OXA-48} gene and IS*1999*, as previously described (17). The chromosomal insertion of bla_{OXA-48} was further mapped by PCR using bla_{OXA-48}-containing DNA fragments purified from pulsed-field gels and primers specific for pOXA-48a. Seventeen primer pairs (Table 2) covering pOXA-48a plasmid backbone were used for PCR mapping; the targets were *trbN*, *pemI*, *mucA*, *nuc*, *orf22*, *orf23*, *orf24*, *korC*, *orf28*, *orf29*, *orf30*, *mobB*, *mobA*, *traJ*, *traU*, *repA*, and *parA*.

Amplification by nested TAIL-PCR. The sites flanking the pOXA-48 insertion site in the *E. coli* chromosome were amplified by a nested thermal asymmetric interlaced PCR (TAIL-PCR) approach. The primer targeting the pOXA-48 insert was ORF-25-R1 (R stands for reverse) (5′-GCCAGC GAGAAGCGAACAAAACG-3′), and the arbitrary degenerate primers were TAIL-pcr-F1 (F stands for forward) (5′-GTAATACGACTCACTAT AGGGCACGCGTGGTNTCGASTWTSGWGTT-3′), TAIL-pcr-F2 (5′-GTAATACGACTCACTATAGGGCACGCGTGGTNGTCGASWGANA WGAA-3′), and TAIL-pcr-F3 (5′-GTAATACGACTCACTATAGGGCA CGCGTGGTWGTGNAGWANCANAGA-3′). The amplifications comprised 15 standard PCR cycles, including 10 high-stringency cycles (annealing temperature, 62°C) and then 5 low-stringency cycles (annealing temperature, 35°C). The PCR product (1 μl) was then amplified under high-stringency conditions by nested PCR (annealing temperature, 62°C) using the primer ORF25-R2 (5′-CGCAACATCAAAACGAGCTCC-3′) and a primer targeting the adaptor part of the degenerated primers (5′-G TAATACGACTCACTATAGGGC-3′).

Plasmid and chromosome sequencing. Plasmid DNA of *Raoultella planticola* strain RA35 was purified with the NucleoBond Xtra Midi kits (Macherey-Nagel) following the manufacturer's instructions. After fragmentation, DNA was sequenced with Roche 454 GS-FLX system. The genomic DNA of *E. coli* EC15 was extracted and purified using the Gentra Puregene Yeast/Bact kit (Qiagen, Valencia, CA) and sequenced using Illumina sequencing technology with 500-bp paired-end libraries.

Bioinformatic analyses. The *de novo* assemblies were performed with MIRA package (*m*imicking *i*ntelligent *r*ead *a*ssembly implemented in Linux) (18). The contiguous sequences (contigs) were then aligned with the pOXA-48a plasmid sequence (JN626286) using the BLAST algorithm (19). The gaps and insertions between contigs were closed or checked by PCR and Sanger sequencing. Open reading frames (ORFs) were predicted and annotated using RATT (20). The resulting annotation was manually checked and curated using Artemis (21). The resulting DNA sequences were compared with the pOXA-48a sequence using Easyfig (22).

TABLE 2 Primers used in this study

Target	Primer sequence (5′ to 3′ end)	Positioning of primers in pOXA-48a sequence	Reference
IS*1999*	CAGCAATTCTTTCTCCGTG	2.780–2.798/7.520–7502[a]	6
	CAAGCACAACATCAAGCGC	3.764–3.746/6.536–6.554[a]	
bla$_{OXA-48}$	AATACACGCATAACGTCCCC	6.232–6.251	32
	GCCATCACAAAAGAAGTGCTC	5.489–5.469	
lysR	GCTAGTGCCAATCTTACAGG	4.983–5.002	10
	GCTGCAAATAGCATCATACC	4.262–4.243	
pemI	TGACCATGCCAACTTCATT	8.022–8.004	This study
	AAACATACCAGCCTTTAACG	7.869–7.850	
	CCCTCCTGACCAGCCGCC	8.163–8.149	
trbB	CACCGCTTCACCAGAAAA	61.698–61.715	This study
trbA	GAAGCGTTCCGAGAATTTG	519–501	This study
trbN	CAGAGAATTTTGGTAAACC	1.314–1.332	This study
	CCGCCCTCAATCCCGCTGC	1.688–1.670	
mucA	CGTTCACTGTCATGAGACAC	8.995–9.014	This study
	GGATCAATACCTTCTCCG	10.203–10.187	
parA	GCAGTGAAAACGTTGATCAG	18.746–18.765	6
	GATCGCAATGCGTCTTGGTG	19.277–19.258	
nuc	GATAAGAATCTGGAAGAAAC	20.047–20.066	This study
	GAGCCTTGATAATACGACG	20.745–20.727	
orf22	CCTGCAGGGCTATTTATTC	22.482–22.500	This study
korC	TTTTGCGCTGCTATTTCC	23.859–23.842	This study
orf25	GGAAATAGCAGCGCAAAA	23.842–23.859	This study
	CCATAACATCGCCATCAT	24.048–24.031	
	ATGATGGCGATGTTATGG	24.031–24.048	
	GGTTCGATGCCCGTATTG	25.237–25.220	
orf28	GACTGGCGCAGGAGTAAA	28.046–28. 063	This study
orf30	GTGGTTCAGGGTGAACAG	29.678–29.661	This study
mobB	TATCTGATGAGCCGTAGC	33.189–33206	This study
mobA	CGCTCTTCCATCGACTTC	34.487–34.470	This study
traJ	GCACCAGGAAGGTTTGAT	37.849–37.866	This study
	CGGGTTCTTTCAGTTGCA	38.251–38.234	
traU	ATCTCACGCAATCTTACGTC	48.129–48.148	6
	TCGCGTCATGCGTGATCTTC	48.705–48.686	
repA	GACATTGAGTCAGTAGAAGG	56.427–56.446	6
	CGTGCAGTTCGTCTTTCGGC	57.351–57.332	

[a] Positioning of primers corresponding to the two copies of IS*1999*.

RESULTS

Genetic background of bacteria. The 22 isolates included in this study produced the carbapenemase OXA-48. The isolates belonged to the *E. coli* (clinical strains [$n = 9$]; colonizing strains [$n = 3$]), *K. pneumoniae* ($n = 5$), *Enterobacter cloacae* ($n = 2$), *Enterobacter aerogenes* ($n = 1$), *Raoultella planticola* ($n = 1$), and *Citrobacter koseri* ($n = 1$) species. The genetic background of *K. pneumoniae* and *E. coli* producing OXA-48 was investigated by MLST (Table 1). Three *K. pneumoniae* isolates (KP9, KP26, and KP112) belonged to sequence type 866 (ST866). The three other *K. pneumoniae* isolates were assigned to ST45 (KP34), ST1156 (KP78), and ST1157 (KP87). Seven STs were represented among *E. coli* isolates (ST227 [$n = 2$], ST617 [$n = 2$], ST38 [$n = 3$], ST88 [$n = 2$], ST131 [$n = 1$], ST1711 [$n = 1$], and ST127 [$n = 1$]), and they covered all *E. coli* phylogroups (Table 1).

Location of *bla*$_{OXA-48}$ in plasmids or chromosome. OXA-48-encoding transconjugants were obtained from 14 strains with OXA-48-encoding genes. Plasmids from natural isolates and their transconjugants were extracted and hybridized with a *bla*$_{OXA-48}$ probe. The results showed that the *bla*$_{OXA-48}$ gene was located on

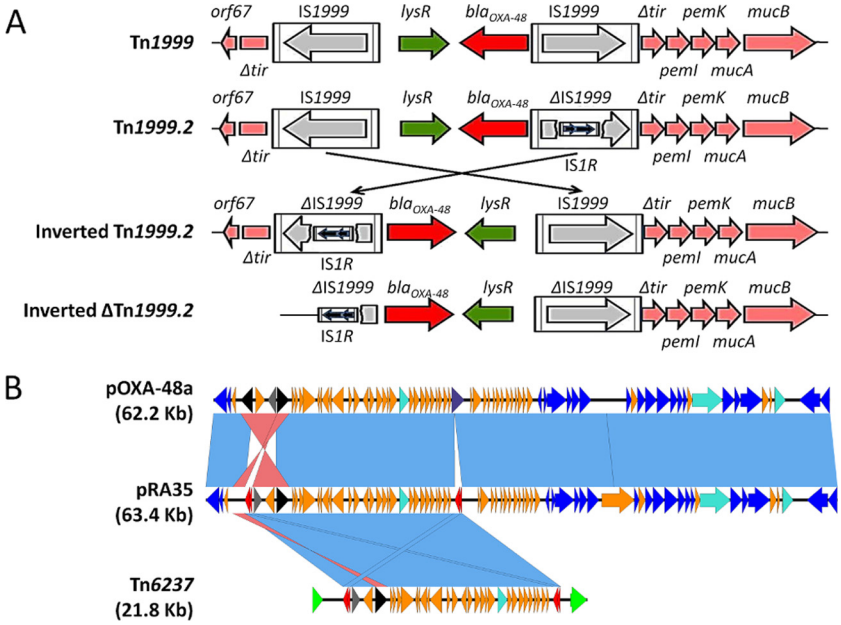

FIG 1 Comparison of pRA35 plasmid and bla_{OXA-48} chromosome-mediated insert (Tn*6237*) with pOXA-48a plasmid. (A) Schematic diagram showing the rearrangements in Tn*1999*-type transposons encoding OXA-48. (B) Global comparison performed using the bioinformatics tools Easyfig. The transposases of insertion sequences are represented by red arrows for IS*1R* and black arrows for IS*1999*, bla_{OXA-48} by gray arrows, genes implicated in transfer and mobility (*mob* and *tra*) by navy blue arrows, genes specific to pOXA-48a-like plasmids (*parA*, *traU*, and *parA*) by light blue arrows, and *E. coli* chromosome genes by green arrows.

~62-kb plasmids (64% [14/22] [Table 1]). Further analysis of these plasmids from transconjugants revealed similar restriction profiles (data not shown). None of the plasmids could be assigned to an incompatibility group by the PBRT method. However, the *repA*, *traU*, and *parA* genes of pOXA-48a were detected in all plasmids, suggesting that the plasmids had an IncL/M pOXA-48a-like backbone.

Despite many attempts, no transconjugants or transformants were obtained from eight unrelated *E. coli* isolates belonging to the A, D, and B2 *E. coli* phylogroups. The insertion of bla_{OXA-48} into the bacterial chromosome was therefore investigated by PFGE after I-CeuI nuclease treatment and hybridization. Distinct DNA fragments, with a range of 600 to 880 kb, hybridized with the bla_{OXA-48} probe (data not shown), suggesting that the insertion of bla_{OXA-48} in *E. coli* chromosomes is not rare (8/22 [36%]) and may occur at different sites.

Genetic environment of bla_{OXA-48}. The bla_{OXA-48} gene was identified as part of the transposons designated Tn*1999* to Tn*1999.4* (6, 9–11). To characterize the genetic environment of bla_{OXA-48} in our strains, we mapped by PCR and sequencing the Tn*1999*-type transposons harboring bla_{OXA-48}. The bla_{OXA-48} gene was predominantly associated with Tn*1999.2* (68% [15/22]). However, the eight bla_{OXA-48} genes on *E. coli* chromosomes (32% [7/22]) were located in incomplete Tn*1999.2* elements, in which the IS*1999* element flanking bla_{OXA-48} was truncated. Finally, bla_{OXA-48} was carried by Tn*1999* in *K. pneumoniae* KP87, and we were unable to identify a Tn*1999*-type element in *E. coli* EC49.

PCR mapping covering the whole sequence of pOXA-48a was then performed by targeting 17 genes and the sequences overlapping Tn*1999.2* edges. The complete backbone of pOXA-48a was observed in all bacteria, except for *E. coli* harboring a chromosome-mediated OXA-48 gene, in which we observed PCR products only for sequences located between bla_{OXA-48} and *orf25* of pOXA-48a. In addition, no amplification was obtained with the primers targeting the sequences overlapping Tn*1999.2* edges for five plasmids and in all *E. coli* harboring a chromosome-mediated OXA-48 gene, suggesting a significant rearrangement in the vicinity of the transposon encoding OXA-48.

Sequence of pRA35 plasmid. To investigate the rearrangement of Tn*1999.2*, we sequenced the ~62-kb plasmid of *R. planticola* RA35(pRA35) using a next-generation sequencing approach. The sequencing reactions of pRA35 plasmid yielded 9,607 reads with an average length of 407 bases. A total of 9,303 reads with an average length of 355 bases were conserved (97%) after trimming, amounting to a total number of 3,302,032 bases of DNA. After contig assembling, gap closing, and repeat sequence mapping by Sanger sequencing, a total of 8,576 reads (92% of reads with an average length of 354 bases) totaling 3,037,308 bases were assembled to obtain the complete sequence of pRA35, resulting in an average of 47× sequence coverage (range, 38× to 121×). Overall, the complete sequencing of pRA35 showed an IncL/M circular plasmid of 63.434 kb with an average G+C content of 51.2%. The pRA35 and pOXA-48a nucleotide sequences shared overall 99% identity, and most of the synteny was conserved. Differences in the DNA sequence were observed in several genes or open reading frames; the most divergent genes were the nuclease-encoding gene and the DNA primase gene. However, the pRA35 plasmid differed from the reference plasmid pOXA-48a in three major respects (Fig. 1): (i) the insertion of an additional copy of the IS*1R* insertion sequence in *orf25*, (ii) the presence of the Tn*1999.2* transposon, which differs from Tn*1999* by the insertion of the IS*1R* insertion sequence in the IS*1999* insertion sequence located upstream of bla_{OXA-48}, and (iii) an inverted orientation of Tn*1999.2*. The orientation of Tn*1999.2* was investigated in all

OXA-48-encoding plasmids by PCR mapping and sequencing. The inverted orientation was detected in five plasmids (5/14 [35%]).

Sequence of the chromosome insert containing the bla_{OXA-48} gene. To understand the insertion of bla_{OXA-48} into the *E. coli* chromosome, the bla_{OXA-48} environment was sequenced in *E. coli* strain EC15 using a next-generation sequencing approach. The sequencing reactions yielded 18,710,176 reads with an average length of 101 bases. A total of 18,441,693 reads of 93-base average lengths were conserved (98.6%) after trimming. Three contigs containing sequences corresponding to pOXA-48a were obtained by *de novo* assembling. After gap closing and repeat sequence mapping by Sanger sequencing, we obtained a contiguous sequence of 27.6 kb, which contained a 21.8-kb central fragment related to the pOXA-48a sequence flanked by 2.7-kb and 3.1-kb *E. coli* chromosome sequences. A total of 112,059 reads (0.6%) with an average length of 93 bases totaling 10,415,879 bases were aligned to 100% of this sequence, resulting in an average of 377× sequence coverage (range, 62× to 795×).

The sequence of the bla_{OXA-48} chromosomal insert shared 100% identity with the pRA35 sequence and identical synteny (Fig. 1). The insert was flanked by two copies of IS*1R*, the first provided by the truncated and inverted Tn*1999.2* transposon, and the second inserted into *orf25*, as observed in the pRA35 plasmid. The deletion in Tn*1999.2* was due to the loss of residual IS*1999* sequences upstream of Tn*1999*.2-located IS*1R*. Between the IS*1R* insertion sequences, after the truncated Tn*1999.2* transposon, we observed 9 genes (*pemI*, *pemK*, *mucA*, *mucB*, *resD*, *parA*, *parB*, *nuc*, and *korC*) and 18 hypothetical ORFs, as previously observed in pRA35 and pOXA-48a. These genes and ORFs, associated with the flanking IS*1R* insertion sequences, formed an IS*1R*-based composite transposon designated Tn*6237*. The flanking regions of this element corresponded to the hypothetical gene *orf33* belonging to the II_{536} pathogenicity island, which has been observed in the reference uropathogenic strain *E. coli* 536 (23). Twelve-base imperfect direct repeats (GATCACTTAGAT and GATCTTTTAGAT) were observed at the site of inversion, suggesting that the insertion of Tn*6237* into the *E. coli* chromosome is the result of a transposition event. PCR mapping of Tn*6237* in *E. coli* showed that Tn*6237* is conserved in all strains harboring a chromosome-mediated OXA-48-encoding gene. However, sequencing of nested TAIL-PCR products revealed distinct insertion sites. In the EC264 strain (ST227, phylogroup A), the bla_{OXA-48} insert was detected in the *yqjF* gene, which encodes a putative quinol oxidase subunit. In *E. coli* EC8 (ST131, phylogroup B2), the bla_{OXA-48} insert was detected in an intergenic region ~375 bp at the 5′ site of the *pgi* gene (glucose-6-phosphate isomerase). Overall, these data showed that the chromosome-mediated bla_{OXA-48} inserts exhibited similar organization in all strains and corresponded to a novel composite transposon, which can be inserted in different sites of the *E. coli* chromosome.

DISCUSSION

Mobile genetic elements, including large broad-host-range plasmids, transposons, and insertion sequences (IS) have played a part in the evolution and generation of diversity of bacterial populations. They performed a critical role in the horizontal and vertical transfer and rapid spread of virulence factors and drug resistance genes in *Enterobacteriaceae*. IncL/M-type plasmids are currently detected worldwide in *Enterobacteriaceae* isolates of different origin and are considered to be epidemic resistance plasmids (24). They were implicated in the large dissemination of specific genes encoding extended-spectrum β-lactamases (ESBL), in particular the $bla_{CTX-M-3}$ gene. IncL/M-type plasmids have also been reported to contribute to the diffusion of the carbapenemase-encoding genes bla_{NDM-1} and bla_{OXA-48}.

In this study, most bla_{OXA-48} genes were also located in ~62-kb IncL/M plasmids related to pOXA-48a, as previously observed in Europe and the Mediterranean region (25). However, most plasmids harbored bla_{OXA-48} inserted into the Tn*1999.2* transposon and not into the Tn*1999* transposon, as previously observed in pOXA-48a. In addition, 67% (8/12) of OXA-48-producing *E. coli* strains investigated in this study harbored a chromosome-mediated gene encoding OXA-48. The chromosome location of bla_{OXA-48} is therefore not rare and is observed in unrelated *E. coli* isolates. This chromosomal location of bla_{OXA-48} was associated with the presence of a truncated form of Tn*1999.2* and significant rearrangement in the vicinity of this element.

To investigate the mechanism underlying the chromosomal location of the OXA-48-encoding gene, we sequenced plasmid pRA35, the chromosomal insert encoding bla_{OXA-48} in *E. coli* EC15 and flanking regions of two additional chromosomal inserts. We observed a 21.9-kb bla_{OXA-48}-encoding and composite transposon designated Tn*6237* in the *E. coli* chromosome and pRA35. This element was detected in all strains harboring an OXA-48-encoding chromosomal insert. It forms an IS*1R*-based composite transposon harboring bla_{OXA-48}, which is responsible for its insertion into the *E. coli* chromosome.

IS*1* is one of the smallest bacterial insertion sequences isolated so far. It is 768 bp long, includes two ~23-bp imperfect inverted repeats (IRL [inverted repeat left] and IRR [inverted repeat right]) located at its ends, and two partly overlapping open reading frames (*insA* and *insB′*) located in the 0 and −1 relative translational phases, respectively (26). IS*1* transposition may appear as a simple insertion or as a replicative fusion event (27, 28). The origin of the second copy of IS*1R* observed in pRA35 and Tn*6237* may therefore be the result of the replicative transposition of the IS*1R* insertion sequence located in Tn*1999.2*.

The IS*1* transposition can occur in both orientations, and the direction of IS*1* is not an important factor in transposition. Reports have mentioned that the gene being mobilized should be flanked by short terminal inverted repeats within the IS*1* sequence regardless of the orientation of the two IS*1* elements (29). The orientation of the IS*1R* elements in Tn*6237* and pRA35 is therefore compatible with the formation of a functional composite transposon. IS*1*-based composite transposons (elements Tn*9* and Tn*1681*) have already been reported (30). In Tn*1681*, the mobile element is flanked by two inverted repeats of IS*1*, which bracket the *E. coli* heat-stable toxin. In Tn*9* and derivatives, a gene encoding chloramphenicol acetyltransferase is bracketed by two direct repeats of the IS*1* insertion sequence, as observed in our bla_{OXA-48}-encoding transposon. In the *E. coli* EC15 chromosome, this IS1*R*-based transposable element is flanked by direct repeats of the insertion site, which may be marks of a transposition event.

Investigation of the target specificity of the IS*1* element indicated that acquisition of IS*1* is generally located within or directly adjacent to the A+T-rich content segments (31). The Tn*6237* insertion site in the EC15 strain is the *orf33* gene of the pathogenicity island (PAI) II_{536}, which has been previously reported in the uropathogenic *Escherichia coli* 536 strain (23). The *orf33* gene (1.668

kb) and surrounding sequence are characterized by a very low-GC percentage (~33%), as at the insertion sites in *E. coli* EC264 (~44%) and EC8 (~37%). Likewise, the IS*1R* insertion sites in the *orf25* gene in pRA35 and Tn*6237* are identical and characterized by a low GC percentage (40%), which makes a putative hot spot of IS*1R* insertion in the backbone of pOXA-48a-like IncL/M plasmids and therefore a region likely to be variable.

In this work, we provide new insights into the bla_{OXA-48} genetic environment and describe its high plasticity, which is promoted by Tn*1999.2* inversion and IS*1R* elements responsible for the transposition of the OXA-48-encoding fragment derived from pOXA-48 in *E. coli* chromosomes.

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We declare that we have no conflicts of interest.

REFERENCES

1. **Paterson DL, Bonomo RA.** 2005. Extended-spectrum beta-lactamases: a clinical update. Clin. Microbiol. Rev. **18:**657–686. http://dx.doi.org/10.1128/CMR.18.4.657-686.2005.
2. **Nordmann P, Dortet L, Poirel L.** 2012. Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol. Med. **18:**263–272.
3. **Nordmann P, Naas T, Poirel L.** 2011. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg. Infect. Dis. **17:**1791–1798.
4. **Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen Ø, Seifert H, Woodford N, Nordmann P, European Network on Carbapenemases.** 2012. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clin. Microbiol. Infect. **18:**413–431. http://dx.doi.org/10.1111/j.1469-0691.2012.03821.x.
5. **Poirel L, Potron A, Nordmann P.** 2012. OXA-48-like carbapenemases: the phantom menace. J. Antimicrob. Chemother. **67:**1597–1606. http://dx.doi.org/10.1093/jac/dks121.
6. **Poirel L, Bonnin RA, Nordmann P.** 2012. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. Antimicrob. Agents Chemother. **56:**559–562. http://dx.doi.org/10.1128/AAC.05289-11.
7. **Berger S, Alauzet C, Aissa N, Hénard S, Rabaud C, Bonnet R, Lozniewski A.** 2013. Characterization of a new blaOXA-48-carrying plasmid in Enterobacteriaceae. Antimicrob. Agents Chemother. **57:**4064–4067. http://dx.doi.org/10.1128/AAC.02550-12.
8. **Espedido BA, Steen JA, Ziochos H, Grimmond SM, Cooper MA, Gosbell IB, van Hal SJ, Jensen SO.** 2013. Whole genome sequence analysis of the first Australian OXA-48-producing outbreak-associated Klebsiella pneumoniae isolates: the resistome and in vivo evolution. PLoS One **8:**e59920. http://dx.doi.org/10.1371/journal.pone.0059920.
9. **Carrër A, Poirel L, Yilmaz M, Akan OA, Feriha C, Cuzon G, Matar G, Honderlick P, Nordmann P.** 2010. Spread of OXA-48-encoding plasmid in Turkey and beyond. Antimicrob. Agents Chemother. **54:**1369–1373. http://dx.doi.org/10.1128/AAC.01312-09.
10. **Giani T, Conte V, Di Pilato V, Aschbacher R, Weber C, Larcher C, Rossolini GM.** 2012. Escherichia coli from Italy producing OXA-48 carbapenemase encoded by a novel Tn1999 transposon derivative. Antimicrob. Agents Chemother. **56:**2211–2213. http://dx.doi.org/10.1128/AAC.00035-12.
11. **Potron A, Nordmann P, Rondinaud E, Jaureguy F, Poirel L.** 2013. A mosaic transposon encoding OXA-48 and CTX-M-15: towards pan-resistance. J. Antimicrob. Chemother. **68:**476–477. http://dx.doi.org/10.1093/jac/dks397.
12. **Beyrouthy R, Robin F, Cougnoux A, Dalmasso G, Darfeuille-Michaud A, Mallat H, Dabboussi F, Hamzé M, Bonnet R.** 2013. Chromosome-mediated OXA-48 carbapenemase in highly virulent Escherichia coli. J. Antimicrob. Chemother. **68:**1558–1561. http://dx.doi.org/10.1093/jac/dkt051.
13. **Diancourt L, Passet V, Verhoef J, Grimont PAD, Brisse S.** 2005. Multilocus sequence typing of Klebsiella pneumoniae nosocomial isolates. J. Clin. Microbiol. **43:**4178–4182. http://dx.doi.org/10.1128/JCM.43.8.4178-4182.2005.
14. **Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MCJ, Ochman H, Achtman M.** 2006. Sex and virulence in Escherichia coli: an evolutionary perspective. Mol. Microbiol. **60:**1136–1151. http://dx.doi.org/10.1111/j.1365-2958.2006.05172.x.
15. **Kado CI, Liu ST.** 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. **145:**1365–1373.
16. **Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ.** 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods **63:**219–228. http://dx.doi.org/10.1016/j.mimet.2005.03.018.
17. **Bonnet R, Marchandin H, Chanal C, Sirot D, Labia R, De Champs C, Jumas-Bilak E, Sirot J.** 2002. Chromosome-encoded class D beta-lactamase OXA-23 in Proteus mirabilis. Antimicrob. Agents Chemother. **46:**2004–2006.
18. **Chevreux B, Wetter T, Suhai S.** 1999. Genome sequence assembly using trace signals and additional sequence information. Proceedings of the German Conference on Bioinformatics, GCB '99, 4 to 6 October 1999, Hannover, Germany.
19. **Zhang Z, Schwartz S, Wagner L, Miller W.** 2000. A greedy algorithm for aligning DNA sequences. J. Comput. Biol. **7:**203–214. http://dx.doi.org/10.1089/10665270050081478.
20. **Otto TD, Dillon GP, Degrave WS, Berriman M.** 2011. RATT: Rapid Annotation Transfer Tool. Nucleic Acids Res. **39:**e57. http://dx.doi.org/10.1093/nar/gkq1268.
21. **Carver T, Berriman M, Tivey A, Patel C, Bohme U, Barrell BG, Parkhill J, Rajandream M-A.** 2008. Artemis and ACT: viewing, annotating and comparing sequences stored in a relational database. Bioinformatics **24:**2672–2676. http://dx.doi.org/10.1093/bioinformatics/btn529.
22. **Sullivan MJ, Petty NK, Beatson SA.** 2011. Easyfig: a genome comparison visualizer. Bioinformatics **27:**1009–1010. http://dx.doi.org/10.1093/bioinformatics/btr039.
23. **Brzuszkiewicz E, Brüggemann H, Liesegang H, Emmerth M, Olschläger T, Nagy G, Albermann K, Wagner C, Buchrieser C, Emody L, Gottschalk G, Hacker J, Dobrindt U.** 2006. How to become a uropathogen: comparative genomic analysis of extraintestinal pathogenic Escherichia coli strains. Proc. Natl. Acad. Sci. U. S. A. **103:**12879–12884. http://dx.doi.org/10.1073/pnas.0603038103.
24. **Bonnin RA, Nordmann P, Carattoli A, Poirel L.** 2013. Comparative genomics of IncL/M-type plasmids: evolution by acquisition of resistance genes and insertion sequences. Antimicrob. Agents Chemother. **57:**674–676. http://dx.doi.org/10.1128/AAC.01086-12.
25. **Potron A, Kalpoe J, Poirel L, Nordmann P.** 2011. European dissemination of a single OXA-48-producing Klebsiella pneumoniae clone. Clin. Microbiol. Infect. **17:**E24–E26.
26. **Hirsch HJ, Starlinger P, Brachet P.** 1972. Two kinds of insertions in bacterial genes. Mol. Gen. Genet. **119:**191–206. http://dx.doi.org/10.1007/BF00333858.
27. **Biel SW, Berg DE.** 1984. Mechanism of IS1 transposition in E. coli: choice between simple insertion and cointegration. Genetics **108:**319–330.
28. **Galas DJ, Chandler M.** 1982. Structure and stability of Tn9-mediated cointegrates. Evidence for two pathways of transposition. J. Mol. Biol. **154:**245–272.
29. **So M, Heffron F, McCarthy BJ.** 1979. The E. coli gene encoding heat stable toxin is a bacterial transposon flanked by inverted repeats of IS1. Nature **277:**453–456. http://dx.doi.org/10.1038/277453a0.
30. **So M, McCarthy BJ.** 1980. Nucleotide sequence of the bacterial transposon Tn1681 encoding a heat-stable (ST) toxin and its identification in enterotoxigenic Escherichia coli strains. Proc. Natl. Acad. Sci. U. S. A. **77:**4011–4015. http://dx.doi.org/10.1073/pnas.77.7.4011.
31. **Zerbib D, Gamas P, Chandler M, Prentki P, Bass S, Galas D.** 1985. Specificity of insertion of IS1. J. Mol. Biol. **185:**517–524. http://dx.doi.org/10.1016/0022-2836(85)90068-3.
32. **Beyrouthy R, Robin F, Cougnoux A, Dalmasso G, Darfeuille-Michaud A, Mallat H, Dabboussi F, Hamzé M, Bonnet R.** 2013. Chromosome-mediated OXA-48 carbapenemase in highly virulent Escherichia coli. J. Antimicrob. Chemother. **68:**1558–1561. http://dx.doi.org/10.1093/jac/dkt051.